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Selenium-Vitamin E Supplementation in Infertile Men

Effects on Semen Parameters and Micronutrient Levels and Distribution

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ABSTRACT

In order to verify the hypothesis that selenium (Se) and vitamin E (Vit E) could improve male fertility, nine oligoasthenoteratozoospermic men were supplemented for a period of 6 mo with Se and Vit E. Compared to the baseline period (presupplementation) of 4 mo, statistically significant increases were observed for Se and Vit E levels, sperm motility, percent live, and percent normal spermatozoa. These improvements are likely to be "supplementation-dependent," since all of the parameters returned to baseline values during the posttreatment period. None of the couples reported a pregnancy during the study. The HPLC analysis conducted on the serum of one of the patients showed the existence of at least six different Se-containing peaks, whose Se content was affected by supplementation. The mechanism(s) involved in these improvements of semen parameters is presently under investigation.

Index Entries: Selenium; vitamin E; infertility.

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INTRODUCTION

Selenium (Se) and vitamin E (Vit E) are now recognized to be important micronutrients in animal reproduction where deficiencies in the male can cause testicular degeneration and severely impair sperm function. Only sparse information is available in the human. We previously reported a significant positive correlation (r = 0.53) between sperm concentration and semen Se in patients consulting for infertility *(1)*. Behne et al. also found a significant correlation (r = 0.59) between semen Se and sperm count, and reported that the Se content of the spermatozoa contributes to this correlation, but other parameters of semen quality were not correlated with Se levels in whole semen or seminal plasma *(2)*. Noack-Füller et al. reached similar conclusions. In addition, they detected a significant correlation (r = 0.465) between semen Se and the percentage of normally formed sperm *(3)*. By analyzing seminal plasma, Xu et al. obtained data showing a significant correlation between Se levels and sperm density in normozoospermic men (r = 0.35), but not in oligozoospermic men *(4)*. Such relationships between Se levels in semen or seminal plasma and sperm concentration or motility were not observed by other investigators *(5,6)*.

In animals, several studies have demonstrated the importance of Se for normal male reproductive function *(7–11)*. The best-characterized effect of Se deficiency on mammalian sperm is an important loss of motility, a breakage at the midpiece level *(10,11)*, and an increased incidence of sperm-shape abnormalities, mostly of the sperm head *(12)*. The role of Se could be mediated via selenoenzymes, such as phospholipid hydroperoxide glutathione peroxidase (PHGPX), recently localized in maturing spermatogenic cells of the rat *(13)*, and glutathione peroxidase found in mouse sperm *(14)*.

Despite the controversy in the literature concerning a possible effect of Se on human male reproduction, we undertook this study where men presenting with the oligoasthenoteratozoospermia (OAT) syndrome were supplemented with Se and Vit E in the hope of improving semen parameters. We included Vit E in the supplementation protocol, because this vitamin is well known to work in synergy with Se as an antiperoxidant *(15,16)* and this vitamin is part of a list of potential drugs for the treatment of male infertility *(17)*.

MATERIALS AND METHODS

Patients

Nine infertile men (mean age: 31.3 yr; range: 28–36) presenting with OAT were enrolled in the study after having signed an informed consent form. Male infertility was confirmed by the complete investigation of

their female partners, who were all normal, except for their postcoital tests, which were abnormal. Each of the couples was reported to be infertile for at least 5 yr.

The patients were examined by a urologist on three visits: a first complete genito-urinary exam was performed prior to the study, whereas a second one was done at midstudy (during the supplementation phase), and the last one was performed at the end of the study. Adverse events and concomitant medication were recorded throughout the study.

Supplementation Protocol

The supplementation protocol was inspired by a previous study *(18)*. Patients received, for the first month of supplementation, 400 mg of Vit E and 100 μg of organic Se daily, in divided doses. For the next 5 mo of supplementation, Se supplementation was doubled to 200 μg daily, and Vit E was kept at 400 mg daily, in divided doses.

Prior to the supplementation phase, the patients were followed for a baseline period of 4 mo, without any supplementation. At the end of the study, a postsupplementation control period of 2 mo was also performed. Patients were asked to visit us on the first Wednesday of each month, from February to January, inclusive.

Se and Vit E Supplements

Supplements given to patients were commercially available in the drugstore (OTC products). Selenium was given as 50 or 100 μg yeast selenium tablets (CE Jamieson and Co Ltd, Ontario, Canada; General Nutrition Center, Québec, Canada and Bio-Santé, Québec, Canada). Vitamin E (*d*-α-tocopherol acetate) was supplied as 200-mg capsules (Pharmacies Jean-Coutu, Québec, Canada; Cumberland Pharmacy, Québec, Canada and Bio-Santé, Québec, Canada).

Study Procedures

On each visit, semen and blood samples were obtained from the patients. Semen was collected by masturbation, in a sterile container, following at least 48 h of sexual abstinence. After liquefaction, a complete semen analysis was performed, according to standard procedures *(19)*. The different tests included physical aspect, volume, pH, sperm concentration, motility, vitality, and sperm morphology.

Immediately following the semen analysis, four aliquots of 40 μL of semen were sampled and kept frozen at –80°C until Se assay. Semen was also centrifuged (13,000*g* for 15 mins), and sperm pellets and seminal plasma aliquots (40 μL) were kept frozen at –80°C until analysis.

Blood was collected in glass vacutainer tubes containing EDTA (for plasma) or no anticoagulant (for serum). Following the hematologic

analysis of each blood sample (on EDTA), four samples of 40 µL each were transferred, as described earlier. All blood samples were centrifuged (1840*g* rpm for 20 mins), serum and plasma were then collected, and four samples of 40 µL of each of these fluids were also transferred for Se assay. The remaining portions of serum and plasma were aliquoted and kept frozen at –80°C.

Se Measurements

Se was measured using a fluorimetric method *(20)*. Briefly, samples and standards (40 µL) were digested in a mixture of nitric-perchloric acid; selenates were reduced to selenites with hydrochloric acid, and a fluorescent complex was formed with 2,3-diaminonaphthalene. In order to minimize intra- and interassay variations, all Se measurements were performed in quadruplicate, and samples from the same patient were all measured together, in a single assay.

Vit E Measurements

α-Tocopherol levels were measured by an HPLC method *(21)*. Briefly, 0.5 mL of standards or plasma samples were mixed vigorously for 15 s with 0.5 mL of ethanol. A 0.5-mL volume of *n*-heptane was then added, and the solution was mixed again. The tubes were centrifuged (700*g* for 5 min), and the organic phase was injected into the HPLC system equipped with a fluorescence detector.

Glutathione Peroxidase Activity Measurements

The glutathione peroxidase (GSH-Px) activity was measured according to the method of Paglia and Valentine *(22)*. Commercial purified GSH-Px from bovine red blood cells (Sigma, St. Louis, MO) was used as standard. Owing to technical problems, only seminal plasma from patient nos. 1–4 inclusive were analyzed for GSH-Px activity.

Hormone Measurements

Commercial radioimmunoassays (RIAs) were used to measure serum testosterone, prolactin, LH (luteinizing hormone), and FSH (follicle stimulating hormone). All measurements were done in duplicate. The hormones were measure before and during the supplementation period.

HPLC of a Patient's Serum

In order to verify the effect of the supplementation on Se-containing proteins, serum samples from one patient (blood taken before, during, and after the period of supplementation) were analyzed by HPLC.

The system was made up of a hydrophobic column (C8, Apex Octyl, 15–20 µ, 8 × 100 mm) with an eluent of trifluoroacetic (TFA) acid 0.1%; a

gradient of isopropanol-acetonitrile (1:1, v:v) was used. The system was eluted at 1.0 mL/min. with proteins detected by absorption at 280 nm; 1-mL fractions were collected. For each sample, 300 µL of serum were diluted in 300 µL of TFA 0.1%; the preparation was filtered through a 0.22-µm filter, and 200 µL of the final preparation were injected into the system.

Statistical Analysis

Statistical analysis of results was done with the Bartlett's chi-square test for variance analysis and Student's *t*-test or Welsh's *t*-test for intergroup comparisons. Analyses were done by comparing all different mean results to the mean of the corresponding first four samples (baseline), obtained during the presupplementation period. Statistically significant results are indicated in Figs. 1–5 with * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.005$, and **** for $p < 0.001$.

RESULTS

The Se supplementation period for the group of patients was from day 0 to day 175.4 (mean), whereas the Vit E supplementation period was from day 0 to day 178.4. No serious adverse events were reported throughout the study. Despite improvements of many semen parameters, no pregnancy was recorded.

Se Levels

During the course of the study, the mean Se levels varied from 62 to 90 ng/mL in semen and from 58 to 82 ng/mL in seminal plasma (Fig. 1). Compared to baseline mean values (semen Se: 74.7 ng/mL and seminal plasma Se: 68.3 ng/mL), only the samples obtained at day 34 showed a statistically significant increase in Se levels both in the semen (86.7 ng/mL) and seminal plasma (78.6 ng/mL). We also observed a statistically significant decrease in semen Se levels at day 96.8 (62.2 ng/mL, Fig. 1A).

Figure 2 presents the mean Se concentrations measured in total blood (Fig. 2A), plasma (Fig. 2B), and serum (Fig. 2C) for all patients. In contrast to the data for semen and seminal plasma, a progressive increase in Se levels in these fluids can be observed. As shown in Fig. 2A, blood Se levels were significantly increased from day 34 to the end of the study, going from a mean baseline value of 171 ng/mL to a maximum of 240 ng/mL. Plasma Se levels (Fig. 2B) were significantly increased at day 34, 66.3 and 125.8, reaching a maximum of 173 ng/mL, compared to a baseline value of 141 ng/mL; serum Se levels (Fig. 2C) also significantly increased during the supplementation period from a baseline value of 144 ng/mL to a maximum of 175 ng/mL at day 152.

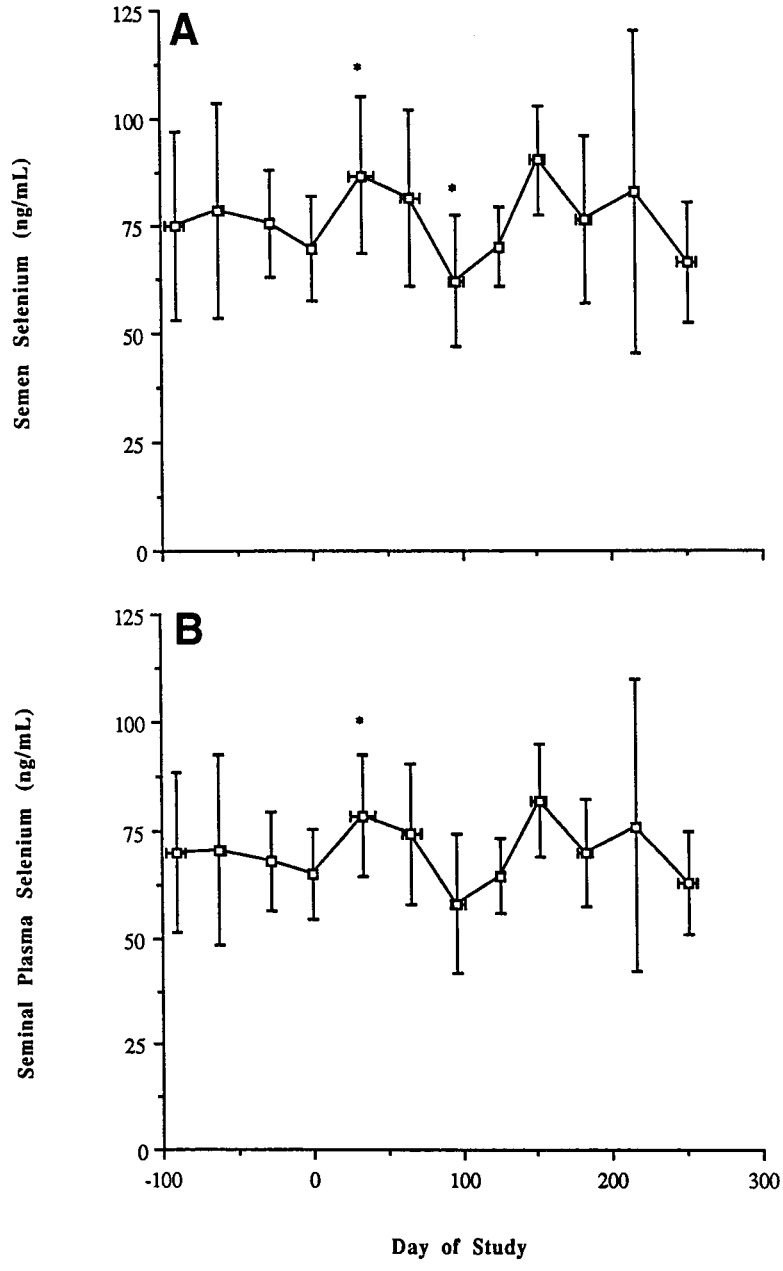


Fig. 1. Semen **(A)** and seminal plasma **(B)** selenium levels during the study (ng/mL, Mean ± SD).

Plasma Vit E Levels

As presented in Fig. 3, plasma Vit E levels showed an impressive increase following the first month of supplementation, going from a mean baseline value of 7.77 μg/mL to a maximum value of 17.5 μg/mL. Plasma Vit E levels were constantly and significantly increased, even during the 2 mo of the postsupplementation period.

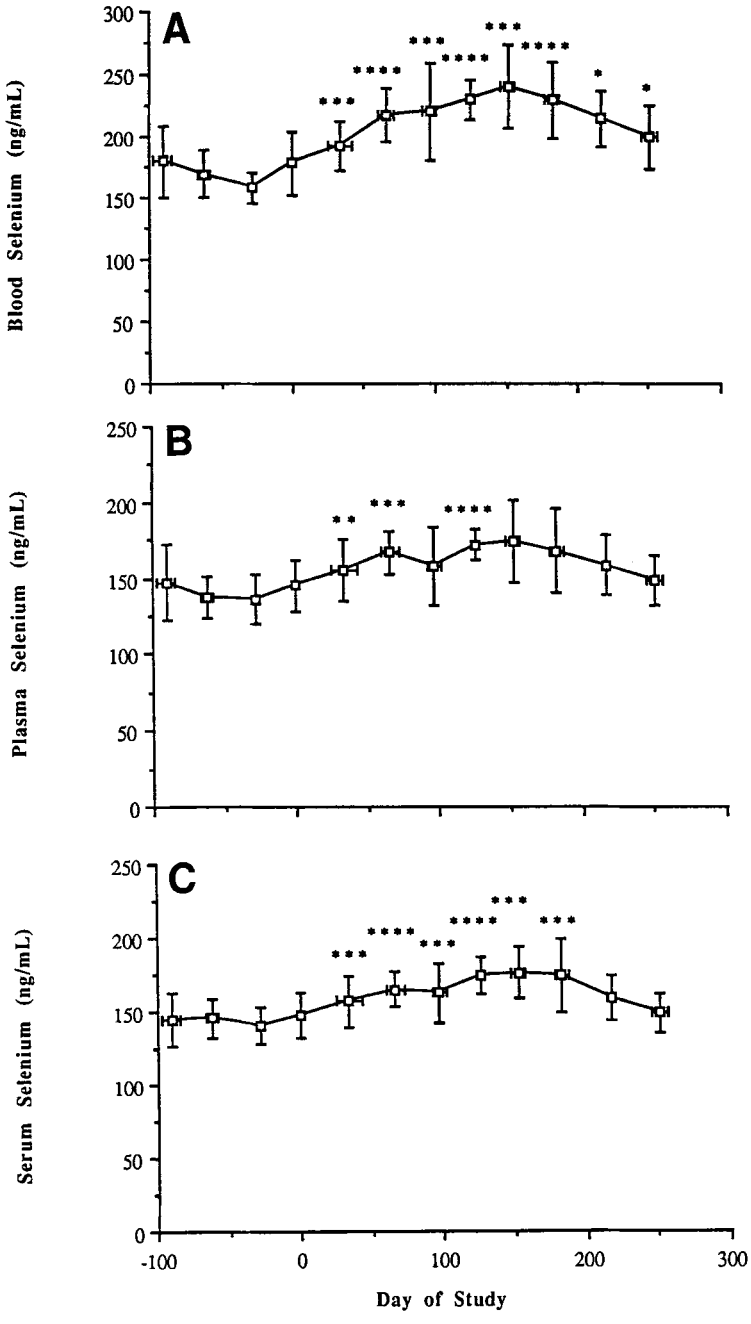


Fig. 2. Blood **(A)**, plasma **(B)**, and serum **(C)** SE levels during the study (ng/mL, Mean ± SD).

Volume of Ejaculate

The mean volume of ejaculate varied between 2.59 mL at day 152 and 3.2 at day –28 (Table 1); this minimum value at day 152 was statistically different from the mean baseline value of 3.11 mL ($p < 0.005$).

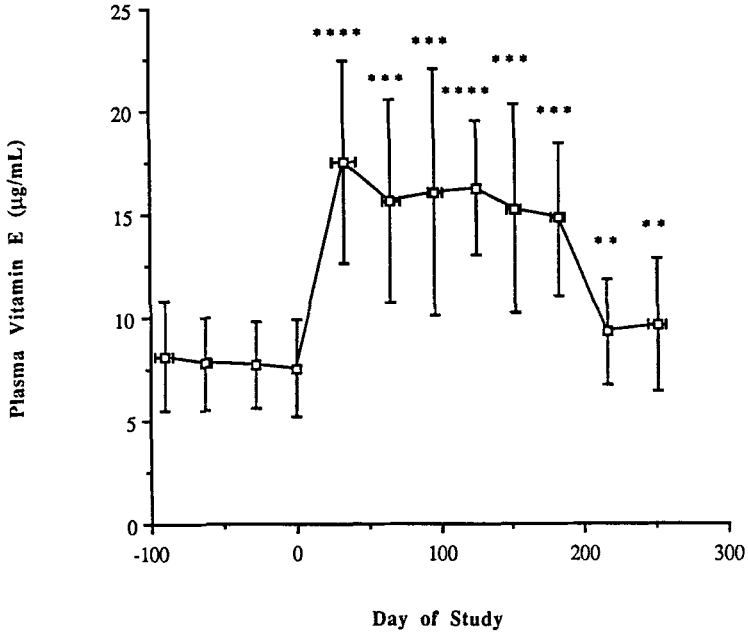


Fig. 3. Plasma Vit E levels during the study (µg/mL, Mean ± SD).

Sperm Concentration

No statistically significant change in sperm concentration was observed, even though a maximum of 14.1 million/mL was reached at day 152 compared to a mean baseline result of 8.2 million/mL (Table 1). Mean total sperm count also showed a maximum at day 152 with 47.1 million sperm/ejaculate; however, this was not statistically different from the mean baseline value of 30 million sperm/ejaculate (data not shown).

Motility

As shown in Table 1, a statistically significant increase in sperm motility was observed at days 34, 96.8, 125.8, 182.7, and 217. A maximum mean value of 31.1% motile sperm was reached at day 182.7 compared to a mean baseline value of 12.1%. The mean total motile sperm counts were also statistically improved at days 96.8, 125.8, and 182.7 (data not shown).

Sperm Morphology

The mean percentages of normal sperm are presented in Fig. 4 (Fig. 4A). Statistically significant increases were observed at days 34, 66.3, 96.8, 125.8, 152, 182.7, and 250.9. From a mean baseline value of 33.6%, a maximum mean value of 62.2% was reached at day 182.7, corresponding to the last day of supplementation. The mean total normal sperm counts

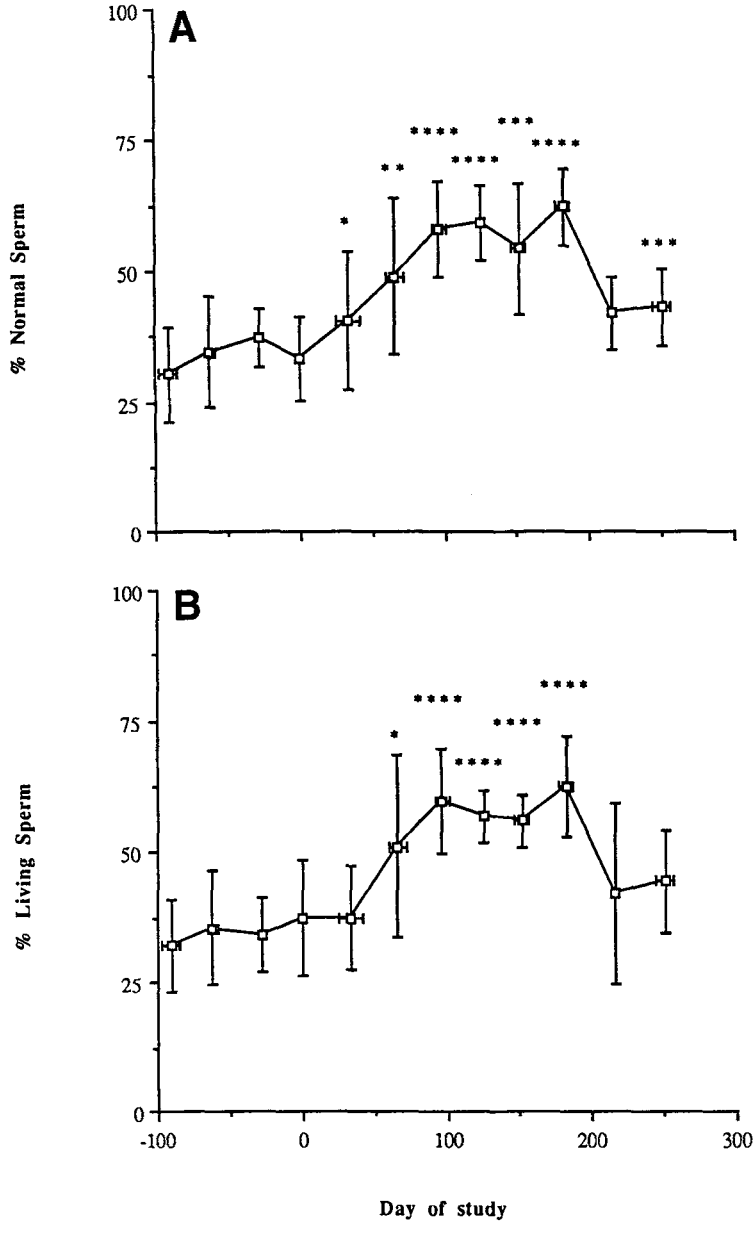


Fig. 4. Percentages of normal **(A)** and live **(B)** spermatozoa during the study (Mean ± SD).

were also significantly increased at days 96.8, 125.8 and 182.7 (data not shown).

Sperm Vitality

The effect of the supplementation on the percentage of live sperm (vital staining) is presented in Fig. 4B. From a mean baseline percentage of 34.8%

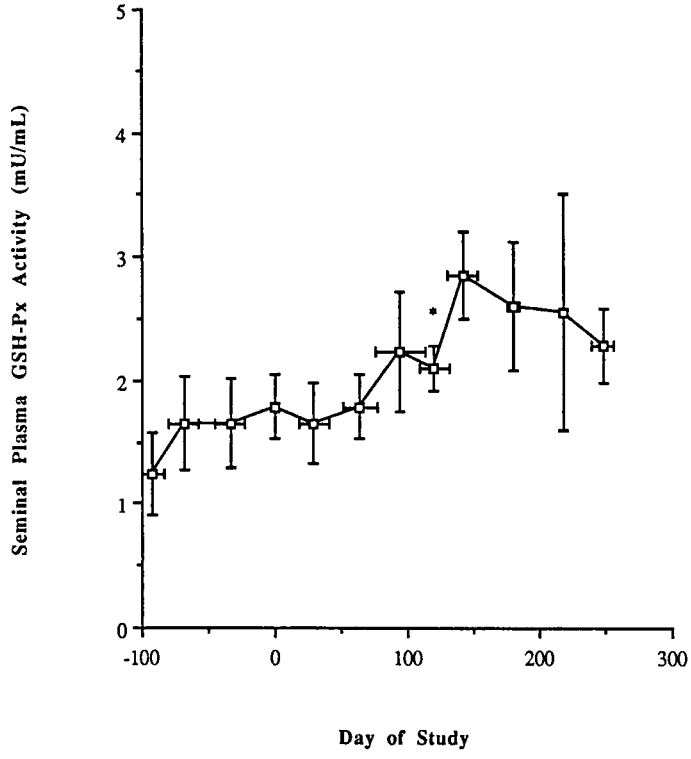


Fig. 5. Seminal plasma GSH-Px activity during the study (Mean ± SD). Note that only samples from Patients nos. 1–4 were analyzed.

obtained before supplementation, the patients reached a maximum of 62.7% at day 182.7, with statistically significant increases at days 66.3, 96.8, 125.8, 152, and 182.7. Furthermore, the mean total live sperm counts were also significantly increased at days 96.8, 125.8, and 182.7 (data not shown).

Seminal Plasma GSH-Px Activity

Figure 5 presents the mean seminal plasma GSH-Px activity observed for the first four patients during the study. A tendency toward an increase in enzyme activity was observed during the supplementation period. Despite the fact that only four patients were analyzed for seminal plasma GSH-Px activity (owing to a technical problem), a statistically significant increase was observed at day 120.3. The maximum GSH-Px activity was measured at day 142 (2.85 mU/mL).

Validation of the Smear Examinations

A second examiner was asked to evaluate the different smears for sperm morphology and vitality performed during the study. Thirty such smears were randomly chosen, and their reference numbers were coded in order to prevent any possible estimation of the dates at which they

Table 1
Semen Characteristics Measured in the Group of Patients During the Study

	Volume, mL		Sperm count, 10^6/mL		Motility, %	
Day ± SD	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
−90.5 ± 6.3	3.07 ± 1.66	0.6–6.3	6.21 ± 6.28	0.9–15.9	11.6 ± 6.4	3–20
−61.7 ± 4.0	3.18 ± 1.50	1.5–5.2	6.89 ± 5.56	0.6–16.2	11.0 ± 11.7	1–40
−28.0 ± 0.0	3.20 ± 1.05	2.2–4.3	11.65 ± 10.55	0.8–32.0	12.9 ± 7.4	3–25
0.0 ± 0.0	3.01 ± 1.05	2.0–5.2	8.73 ± 8.30	3.0–13.8	13.1 ± 12.9	3–45
34.0 ± 8.6	2.72 ± 1.06	1.7–4.9	11.52 ± 11.06	1.0–33.0	18.1 ± 10.9[a]	3–40
66.3 ± 6.1	2.96 ± 2.02	1.4–7.9	10.26 ± 8.26	0.6–27.7	11.6 ± 12.0	2–40
96.8 ± 5.0	2.63 ± 1.53	1.5–5.2	12.44 ± 12.67	1.6–40.5	20.1 ± 14.2[a]	1–50
125.8 ± 3.0	3.18 ± 1.67	1.5–6.8	11.81 ± 10.69	1.4–30.6	23.6 ± 7.9[b]	15–40
152.0 ± 4.9	2.59 ± 1.67[b]	1.1–5.3	14.10 ± 10.66	4.0–29.4	19.0 ± 10.4	10–40
182.7 ± 5.1	2.73 ± 1.43	1.6–6.2	9.39 ± 5.12	5.6–13.2	31.1 ± 14.3[b]	20–55
217.0 ± 0.8	3.01 ± 1.42	1.3–5.0	10.90 ± 6.22	3.4–19.2	16.9 ± 8.2[a]	10–35
250.9 ± 6.0	3.07 ± 2.10	0.9–6.3	10.30 ± 7.87	1.0–17.9	14.2 ± 6.7	3–25

[a] $p < 0.05$.
[b] $p < 0.005$.

were performed. Statistically significant correlations ($p < 0.05$) were obtained between the two examiners, both for normal and viable sperm (data not shown).

Control Population

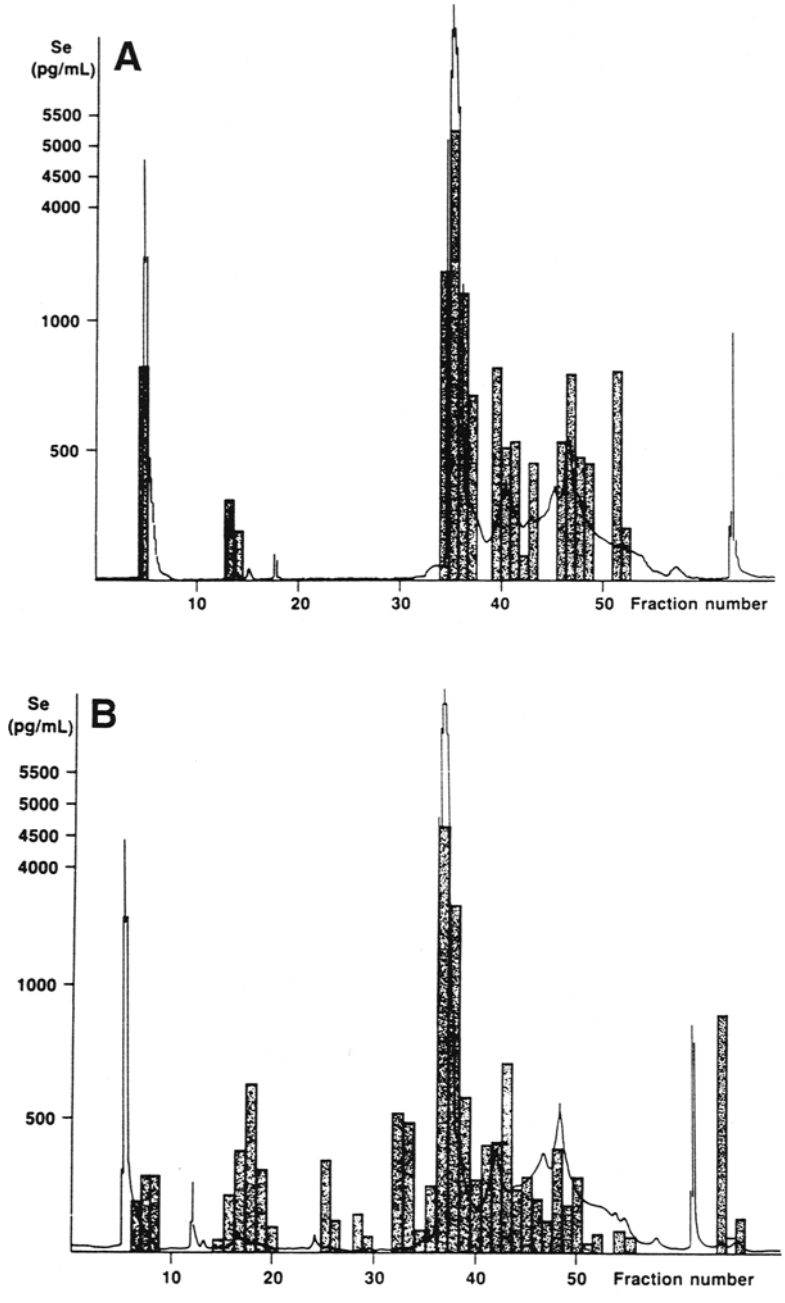
Patients consulting for infertility at our clinic during the same months when this study was done were randomly chosen as control population: the first 15 men of each month were chosen. The same parameters as previously described were measured (except GSH-Px activity and Vit E measurements). No statistically significant changes in these parameters were observed during these 12 mo (data not shown).

Hematology and Hormonal Measurements

The major hematologic measurements (hemoglobin, hematocrit, erythrocytes, leukocytes, and platelets) were not modified by the supplementation protocol (data not shown). No significant changes were observed for serum testosterone, prolactin, LH, or FSH (data not shown).

HPLC of Human Serum

Figure 6 presents the elution profiles of human serum on the hydrophobic column (HPLC-C8). Three different samples from the same



patient were analyzed: prior to the supplementation period (Fig. 6A, day –63), at the end of supplementation (Fig. 6B, day 182), and 2 mo post-supplementation (Fig. 6C, day 252).

For all chromatograms, seven peaks were observed with UV detection at 5, 14, 36, 42, 46, 48 and 64 min. If we compare the Se content of

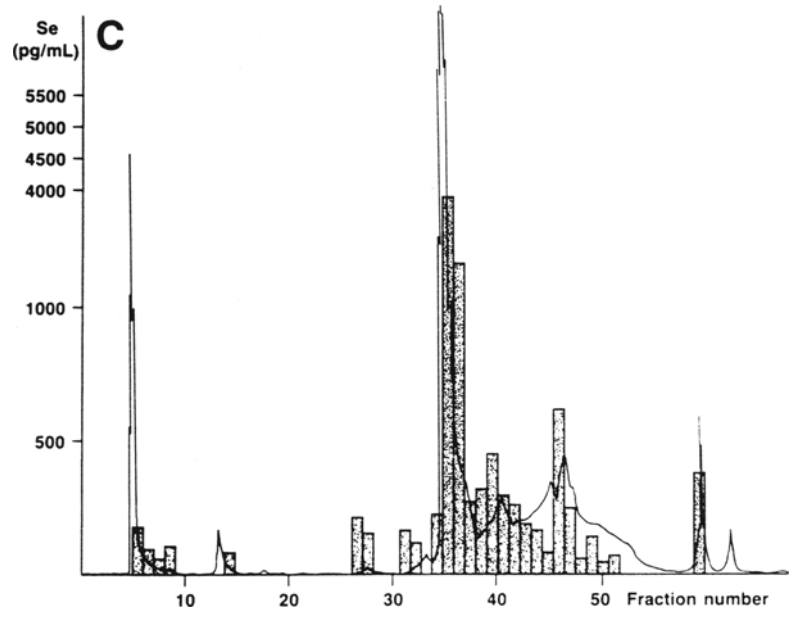


Fig. 6. HPLC of serum samples from a single patient before **(A)**, during **(B)** and following **(C)** the supplementation period. Bars represent Se measured in each fraction (pg/mL).

the fractions, the first sample (Fig. 6A) presents six peaks, the second one (Fig. 6B) eight peaks, whereas the third one (Fig. 6C) presents seven peaks. All elution profiles showed a high similarity in the UV absorption pattern. Overall, a mean recovery of 93.8% of injected Se was obtained.

DISCUSSION

The Se-Vit E supplementation protocol that was used resulted in statistically significant increases in semen, seminal plasma, blood, plasma, and serum Se levels and in Vit E levels in plasma. The patterns of increment are comparable to those reported in other studies *(18,23–25)*. However, comparisons are difficult to make because of the fact that most published studies concerning Se-Vit E supplementation were done in low-Se areas, such as Finland. Furthermore, the supplementation protocols differ slightly from one study to another. Surprisingly, we observed a statistically significant decrease in semen Se levels at day 96.8 (Fig. 1, Panel A); this result was not observed in other studies performed in animals *(9,26–28)*. Although these studies were done with severely Se-deficient animals, our patients were not chosen on the basis of their Se status, but rather because of their primary infertility associated with OAT syndrome.

The volume of the ejaculate was significantly decreased at day 152 (Table 1); this could explain why the maximum mean sperm count was observed on the same day. The total sperm count was not changed, indicating that the rate of sperm production would not be influenced by the supplementation. The control population we used did not show any significant improvement either in sperm concentration, motility, percent normal, or percent live sperm (data not shown).

Sperm motility was significantly improved. This result is in agreement with many previous studies performed in animals *(8,10,29,30)*. In fact, the most characterized effect of a severe Se deficiency on mammalian sperm is a dramatic loss of motility. It is also interesting to note that sperm motility is one of the most reproducible (intra-assay) parameters in semen analysis *(31,32)*; these observations reinforce our results by excluding the possibility of natural intravariability.

The percentages of normal and live sperm were also statistically increased during the supplementation period (Fig. 4). For both parameters, improvements became evident after 2 mo of supplementation with 200 μg of Se plus 400 mg of Vit E daily. Interestingly, this 2-mo period corresponds to the duration of spermatogenesis, suggesting an effect of the supplementation on these parameters.

The increase in mean seminal plasma GSH-Px activity may explain, at least in part, the improvements in semen quality observed during the supplementation period. It was shown that human seminal plasma contains low levels of GSH-Px activity *(33)*; a supplementation with Se might increase this enzymatic activity (Fig. 5) giving an improved protection against lipid peroxidation. In animals, many reports have implicated spontaneous lipid peroxidation as a deleterious factor that affects sperm cell integrity *(14,34–36)*. In the human, the generation of reactive oxygen species has been linked to oligozoospermia *(37–39)*, and evidence has been presented for a possible role of lipid peroxidation in infertility *(40–42)*. The small number of patients in whom seminal plasma GSH-Px activity was measured limits the interpretation of our results.

Vit E could also have a beneficial effect on semen parameters, since a deficiency in this vitamin has been associated with testicular degeneration in the rat, monkey, dog, rabbit, guinea pig, and mouse *(43)*. This author has also reported that Vit E-deficient hamsters show impaired spermatogenesis and that a daily oral supplementation of *d*-α-tocopherol caused the reappearance of spermatozoa in the epididymis.

In the HPLC analysis of serum samples, an overall mean recovery of Se of 93.8% was obtained; this confirms the validity of the method we used to measure Se and allows the comparison of the three different elution profiles. The supplementation protocol did not change the elution profile as monitored by UV absorption; however, the distribution of Se appeared to be modified: six peaks of Se were detected in the serum prior to supplementation, eight during supplementation, and seven in the postsupplementation period (Fig. 6A–C). Some of these peaks are

common to all three samples (peaks at or around 6, 36, and 43 mins); however, their Se contents differ. During supplementation, Se-containing peaks appeared at 25 and 64 min whereas other peaks have increased Se contents compared to the pre- and postsupplementation periods. These data are in support of previous studies concerning the existence of several Se-containing proteins in mammals *(44–48)* that could be influenced by the diet.

As reported by others *(49)*, the supplementation had no effect on hematologic parameters, such as the number of leukocytes, erythrocytes, or platelets, or hemoglobin and hematocrit.

It is acknowledged that this study has certain limits and should be interpreted with caution. Three major sources of variation are liable to cause a spurious association between the Se-Vit E supplementation and the improvement observed in semen parameters. First, it is now well established that seasonal changes in human semen characteristics do occur. The lowest values for sperm concentration have been found in July/August/September, whereas maximum values occur during late winter to early spring *(50–56)*. All of these data were obtained from studies conducted in cities in the northern hemisphere, at latitudes not too distant from that of Montreal. The statistically significant improvements that occurred during supplementation in the present study took place during that period of the year when the lowest values were theoretically expected. Therefore, our results suggest that seasonal changes do not explain the responses observed in this study.

The second major concern is the tendency for semen quality to improve with elapsed time following inclusion into a protocol. Thus, Baker et al. *(57)* have reported that as tests were repeated, there were significant increases in sperm concentration and motility in a group of asthenozoospermic patients. According to these investigators, these changes may result from a selection bias: many of these patients, in followup or treatment, whether efficacious or not, were more likely to show improvement in semen parameters. In our study, the nine subjects had been investigated in our clinic over a period of several years and were consistently found to be oligoasthenoteratozoospermic despite repeated tests. The 4-mo baseline period and the 2-mo posttreatment followup period were chosen to take this possibility into account.

The third source of variation, namely a possible placebo effect, is an important limitation. A wealth of medical therapies have been advocated for male infertility. However, very few forms of treatment have withstood the test of the double-blind placebo-controlled study design. The most extensively documented therapeutic drug in this regard is certainly clomiphene citrate. If we assume that clomiphene is not a useful drug in the treatment of male infertility, as demonstrated in a placebo-controlled trial *(58)*, we are led to conclude that the improvement in semen parameters in individual patients reported in several studies using this medication results from seasonal changes, elapsed time (repeated tests), and

the placebo effect. The study of Sokol et al. *(58)* included a whole year of treatment with placebo or clomiphene; when seasonal changes and the elapsed time effect were taken into account, the authors did not observe any effect of the placebo on semen parameters. In the present study, the improvements in some of the semen parameters are statistically significant and likely to be supplementation-dependent, since a return to baseline values occurred on cessation of the supplementation. Based on this rationale, it can be speculated that the improvement in sperm quality that occurred during supplementation is not likely to be a placebo effect. However, such factors as our approach to the patient and the manner in which the study was conducted could be responsible for a stronger placebo effect. Only a randomized double-blind placebo-controlled trial could adequately answer this question.

SUMMARY

A group of nine infertile men supplemented with Se and Vit E showed statistically significant improvements in their sperm motility, vitality, and morphology compared to a presupplementation control period. Return to baseline values after treatment indicated that the improvements were supplementation-dependent. Prevention of peroxidative damages by selenoenzymes and Vit E could be an underlying mechanism.

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